TAM receptor signaling in development

TAL BURSTYN-COHEN*

The Institute for Dental Sciences, Faculty of Dental Medicine, The Hebrew University, Jerusalem, Israel

ABSTRACT TYRO3, AXL and MERTK comprise the TAM family of receptor protein tyrosine kinases. Activated by their ligands, protein S (PROS1) and growth-arrest-specific 6 (GAS6), they mediate numerous cellular functions throughout development and adulthood. Expressed by a myriad of cell types and tissues, they have been implicated in homeostatic regulation of the immune, nervous, vascular, bone and reproductive systems. The loss-of-function of TAM signaling in adult tissues culminates in the destruction of tissue homeostasis and diseased states, while TAM gain-of-function in various tumors promotes cancer phenotypes. Combinatorial ligand-receptor interactions may elicit different molecular and cellular responses. Many of the TAM regulatory functions are essentially developmental, taking place both during embryogenesis and postnatally. This review highlights current knowledge on the role of TAM receptors and their ligands during these developmental processes in the immune, nervous, vascular and reproductive systems.

KEY WORDS: *TAM receptor, TAM signaling, PROS1, Protein S, GAS6*

Introduction to TAM functions

The impact of the TAM family of receptor protein tyrosine kinases (RTKs) on physiology recently gained momentum, as they play pivotal roles in several physiological systems. TYRO3, AXL and MERTK comprise the TAM family of RTKs, and together with their cognate ligands Growth Arrest-Specific 6 (GAS6) and Protein S (PROS1) mediate various molecular signaling pathways and cellular functions, mostly impacting adult tissues. The two chief functions mediated by TAM receptors in adult tissues are the phagocytic uptake of membranes and apoptotic cells, and negative regulation of the immune response (Lemke, 2013). Indeed, TAMs are expressed by professional and non-professional phagocytes in several tissues where they mediate efferocytosis - the process of phagocytic uptake and clearance of apoptotic cells. Removal of apoptotic germ cells and extruded cytoplasm by Sertoli cells in the testis (Lu *et al.*, 1999), efferocytosis by macrophages of the immune system (Anderson *et al.*, 2003) and by brain microglia during adult neurogenesis (Fourgeaud *et al.*, 2016), as well as bone resorption by osteoclasts (Nakamura *et al.*, 1998) are all mediated by TAM signaling (Lemke *et al.*, 2010; Lu *et al.*, 2010). An exception to efferocytic phagocytosis is seen in the retina, where MER is necessary for uptake of *viable* photoreceptor outer segments by retinal pigment epithelium (RPE) (Burstyn-Cohen *et al.*, 2012; D'Cruz *et al.*, 2000; Duncan *et al.*, 2003; Lu *et al.*, 2010; Prasad *et al.*, 2006). Thus, as part of the "sanitary department", TAMs are pivotal in the rapid clearance of cellular debris, preventing the cells'

secondary necrosis and release of intracellular antigens and DNA which provoke inflammation and autoimmunity (Elliott *et al.*, 2010), ultimately leading to failed tissue homeostasis and pathological conditions such as local or systemic inflammation, autoimmunity and cancer (Poon *et al.*, 2014). In keeping with a role in maintaining tissue homeostasis through phagocytosis, mice lacking TAM receptor function develop autoimmune disease (Lu *et al.*, 2001), male infertility (Lu *et al.*, 1999) and blindness (Duncan *et al.*, 2003; Lu *et al.*, 1999). Another central function fulfilled by TAM receptor signaling is their regulatory role in attenuating inflammatory reactions and immune responses. As negative regulators of immunity, TAMs are expressed by a myriad of immune cell populations, where they specifically restrain innate immunity and the secretion of pro-inflammatory cytokines (Carrera Silva *et al.*, 2013; Rothlin *et al.*, 2015; Rothlin *et al.*, 2007). Thus, TAM-mediated homeostatic regulation and diminution of inflammation is brought about by both the active removal of potential stimulators of auto-immune response, as well as by molecular inhibition of inflammation and immune cell activation (Lemke *et al.*, 2008; Rothlin *et al.*, 2015).

While TAM loss-of-function underlies dysregulated tissue homeostasis as mentioned above, it should be mentioned that the overexpression and upregulation of TAMs is recognized in vari-

---

Abbreviations used in this paper: DKO, double knockout; GAS6, growth-arrest-specific 6; GnRH, gonadotrophin releasing hormone; NSC/NPC, neural stem cell/neural progenitor cell; PROS1, Protein S; RPE, retinal pigment epithelium; RTK, receptor tyrosine kinase; TAM, Tyro3, Axl, Mertk.

---

*Address correspondence to: Tal Burstyn-Cohen. The Institute for Dental Sciences, Faculty of Dental Medicine, Hebrew University-Hadassah, POB 12272, Jerusalem 9112-102, Israel. Tel: +972.2.6758582. Fax: +972.2.6757278. E-mail: talbu@ekmd.huji.ac.il - web: www.tbclab.com - (iD) http://orcid.org/0000-0002-2324-2921

ous human cancers, where activated TAM signaling functionally supports increased proliferation, migration, invasion, enhanced tumor cell survival, angiogenesis and promotes resistance to chemotherapeutic drugs (Axelrod *et al.*, 2014; Cummings *et al.*, 2013; Graham *et al.*, 2014). To date, among TAMs, AXL overexpression was shown to drive chemoresistance in several cancer models (Brand *et al.*, 2014; Elkabets *et al.*, 2015). Thus, taming TAM signaling is critically important for maintaining healthy tissue homeostasis and TAM activation by their ligands PROS1 and GAS6 must also be kept in check.

For many years, the lack of tools to study the roles of the individual TAM receptors and ligands in a cell-specific manner has hampered the understanding of TAM-mediated signaling, especially as different TAM receptor-ligand interactions mediate diverse signaling pathways and altered cellular functions (Graham *et al.*, 2014; Lemke, 2013; Zagorska *et al.*, 2014). The role of GAS6 and PROS1 as TAM agonists was studied *in vitro* for many years, where the role of PROS1 as a functional TAM ligand was debatable at times. It was only following the development of an organotypic *in vitro* system (Prasad *et al.*, 2006) and the generation of a conditional knockout mouse model for *Pros1* (Burstyn-Cohen *et al.*, 2009) that enabled the uncovering of PROS1 functions as a TAM ligand in may tissues *in vivo* (Burstyn-Cohen *et al.*, 2009; Burstyn-Cohen *et al.*, 2012; Carrera Silva *et al.*, 2013).

This review highlights previously identified and newly emerging roles of the TAM receptors and their ligands (Fig. 1) during embryonic and postnatal development. The relevance of TAM signaling components to developmental processes and its relation to disease is discussed.

A brief history of the cloning and identification of TAM receptors and their ligands

The receptors TYRO3, AXL and MERTK were independently cloned by several labs in the early 1990's (Graham *et al.*, 1994; Lai *et al.*, 1994; Lai *et al.*, 1991; O'Bryan *et al.*, 1991), and their ligands identified as PROS1 and GAS6 several years later (Mark *et al.*, 1996; Nagata *et al.*, 1996; Ohashi *et al.*, 1995; Stitt *et al.*, 1995). However, several laboratories reported the *in vitro* stimulation of TAM receptors only by GAS6 and not by PROS1 in various experimental systems (Chen *et al.*, 1997; Godowski *et al.*, 1995; Hall *et al.*, 2001; Mark *et al.*, 1996; Nagata *et al.*, 1996; Ohashi *et al.*, 1995), thus questioning the biological role of PROS1 as a functional TAM ligand. It was not until 2003 when Anderson *et. al.* reported that serum-derived PROS1 stimulated phagocytosis of apoptotic cells by macrophages (Anderson *et al.*, 2003), which was already known to be mediated by MERTK (Scott *et al.*, 2001). A couple of years later PROS1 was reported to stimulate the phagocytic uptake of photoreceptor outer segments by cells of the retinal pigment epithelium (RPE) (Hall *et al.*, 2005;

Prasad *et al.*, 2006). Another line of evidence implying PROS1 as an *in vivo* TAM ligand stemmed from analysis of two independently generated GAS6 null mouse models (Angelillo-Scherrer *et al.*, 2005; Yanagita *et al.*, 2002). Both GAS6 null strains appeared healthy and were not blind due to photoreceptor degeneration as in *Mer*^{-/-} mice (Duncan *et al.*, 2003). Moreover, *Gas6*^{-/-} mice did not present with autoimmune disease or male sterility, as do mice lacking all three TAM receptors (Lu *et al.*, 1999; Lu, Lemke, 2001). The generation and analysis of conditional knockout mice for *Pros1* (Burstyn-Cohen *et al.*, 2009) and ablation of *Pros1* expression in the retina demonstrated the role of PROS1 as an *in vivo* ligand for MERTK in mediating the phagocytosis of photoreceptor outer segments by RPE cells (Burstyn-Cohen *et al.*, 2012). The role
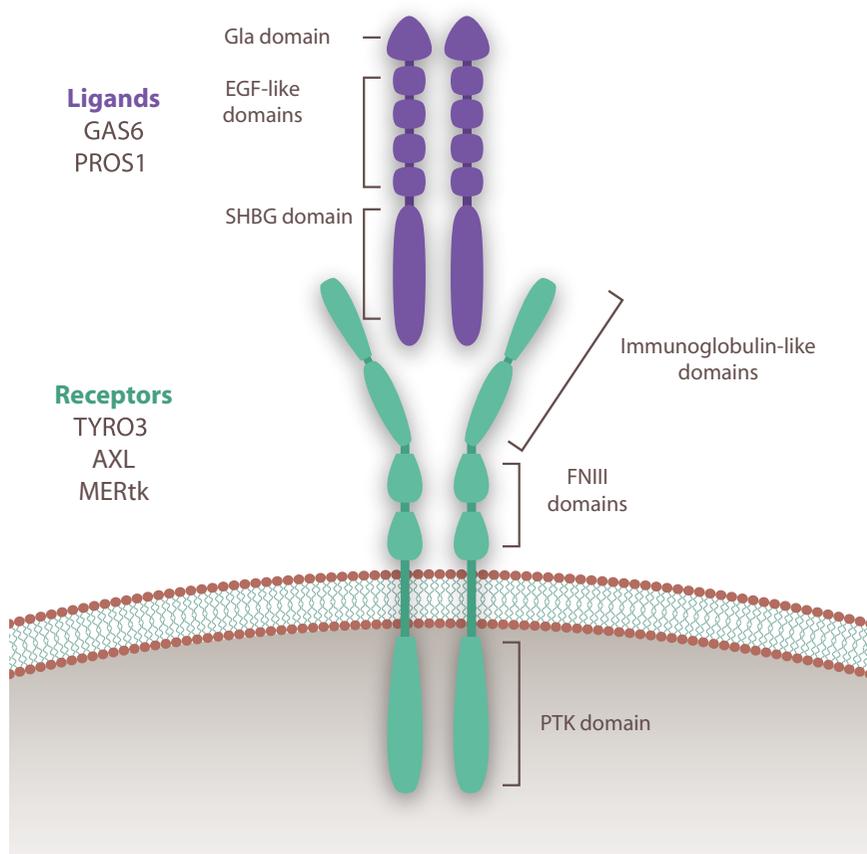

Fig. 1. Structure of the TAM receptor signaling components. *The TAM family of receptor tyrosine kinases is comprised of TYRO3, AXL and MERTK. All receptors (shown in green) share a similar domain structure. The extracellular domain is comprised of two immunoglobulin (Ig) type domains at the amino-most terminus. Two fibronectin type III domains are located adjacent to the transmembrane domain. Their kinase domain resides intracellularly, mediating various intracellular pathways, including PI3K/AKT, ERK/MAPK and JAK/STAT. The ligands GAS6 and PROS1 (shown in purple) are highly homologous, with similar domain structure and 44% amino acid identity (MANFIOLETTI et al., 1993). A gamma-carboxy glutamic acid (Gla) domain is located at the amino-terminus, followed by four EGF-like domains. The carboxy-terminal end of the ligands contains two laminin G type domains, which together comprise the sex hormone binding globulin (SHBG) domain. Gamma carboxylation of the Gla domains is vital for ligand bioactivity, and is a vitamin K-dependent reaction. Thus, ligand activity is sensitive to vitamin K levels and inhibitors. The Gla domain may also bind phosphatidylserine moieties exposed on the surface of apoptotic cells and membrane discs of photoreceptors. Receptor dimerization and activation of the tyrosine kinase domain are induced following ligand binding via SHBG domain - Ig domains interaction.*

of PROS1 as a TAM ligand *in vivo* has since been shown in the adult mouse testis (Lew *et al.*, 2014), and in the immune system (Carrera Silva *et al.*, 2013).

TAM signaling in development

Overlapping expression and functional redundancies between the TAM receptors prompted the generation of mice in which all three TAM receptors were inactivated (Lu *et al.*, 1999). Simultaneous inactivation of all TAM receptors does not result in embryonic lethality, indicating their regulatory roles are dispensable for development (Lemke, 2013; Lu *et al.*, 1999). However, expanding data suggest the TAMs do regulate developmental processes, as TAM receptors and their ligands are expressed by embryonic stem cells and during development in multiple cell types, and inactivation of TAM receptors or their ligands affects normal embryonic and postnatal developmental processes. The biologic functions of TAM receptors and their ligands in maintaining a healthy homeostasis *in adult tissues* has been briefly mentioned above, and was recently extensively reviewed (Graham *et al.*, 2014; Lemke, 2013; Lemke, Burstyn-Cohen, 2010; Rothlin *et al.*, 2015; Rothlin *et al.*, 2010). Here, I will focus on the newly emerging roles of TAM signaling in developmental processes.

Notably, the expression of TYRO3 was first reported to be enriched in embryonic developing mouse tissue, and was therefore initially named Developmental Tyrosine Kinase - DTK (Crosier *et al.*, 1994). The expression pattern of DTK -now formally renamed TYRO3 - was highly enriched in embryonic tissues, including undifferentiated mouse embryonic stem (ES) cells and in various embryonic tissues. In contrast to embryonic tissue, TYRO3 expression in adult tissues was restricted to portions of the gastrointestinal, nervous and reproductive systems (Crosier *et al.*, 1994). The embryonic expression of TYRO3 notwithstanding, to date no gross developmental defects have been attributed to the loss of TYRO3 expression. This may be explained by the overlapping and compensatory roles of TAM receptors demonstrated in various cell types, often with an increase in phenotype severity caused by loss of each additional TAM allele (Lemke, 2013; Lu, Lemke, 2001).

TAM receptor function in the developing reproductive system

Gonadotropin-releasing hormone (GnRH) neurons participate in the hypothaloamic-pituitary-gonadal axis which regulates many aspects of sexual development and maturation in both males and females. In adult rodents and higher mammals, GnRH neurons reside in the hypothalamus, where they secrete GnRH in a pulsatile manner into the pituitary portal system (Fig. 2). In turn, uptake of GnRH by the pituitary initiates secretion of the pituitary gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the circulation. LH and FSH stimulate the gonads to promote gametogenesis and steroidgenesis. In males, LH stimulates the production and secretion of testosterone and android-binding protein by Leydig and Sertoli cells of the testis. In females, LH stimulates the ovarian secretion of androgens, as well as secretion of progesterone at later stages. FSH stimulates Sertoli cells to promote the proliferation and post-mitotic maturation of male germ cells, and the production of estradiol by ovarian granulosa cells in females. Deficiencies in GnRH culminate in the failure to undergo normal pubertal development (Herbison, 2016; Wierman *et al.*, 2011).

During embryogenesis, GnRH neurons undergo extensive migration from their birth location in the olfactory placode/vomeronasal organ to the hypothalamus (Fig. 2). In mice GnRH neurons engage in migration at embryonic day 10.5 (E10.5), when they leave the olfactory placode, migrate along the vomeronasal nerve and traverse into the developing forebrain through perforations at the cribriform plate between E12-E17. Once in the forebrain, GnRH neurons change their migratory trajectory and turn caudally, until they arrive at their final destination – the hypothalamus. GnRH neurons further disperse within this region, and finally send projections to the median eminence, activating pituitary gonadotropes. The defective production or incorrect migration of GnRH neurons underlies reproductive disorders, such as hypogonadotropic hypogonadism as in Kallmann's syndrome (Herbison, 2016; Wierman *et al.*, 2011).

Studying the molecular mechanisms underlying the complex migratory pathway of GnRH neurons has revealed a role for GAS6/AXL signaling in their guidance, proliferation and survival. The elevated expression of AXL by migratory GnRH neuron cell lines compared to post-migratory GnRH neurons suggested its involvement in GnRH migration (Fang *et al.*, 1998). AXL stimulation by GAS6 in the Gn10 migratory cell line induced activation of extracellular signal-regulated kinase (ERK) and Akt in a GAS6-dependent fashion, resulting in increased cell survival and protection from apoptotic cell death (Allen *et al.*, 1999). The expression of all TAM receptor signaling components was later identified to be differentially expressed, with *Axl* and *Tyro3* predominantly expressed among low GnRH expressing migratory GnRH neurons. By contrast, *Mer* was upregulated in high GnRH expressing post-migratory neurons, further suggesting their dynamic role in GnRH development (Pierce *et al.*, 2008).

In vitro silencing of *Axl* and *Tyro3* identified their role in protection of migratory GnRH neuronal cells from apoptosis induced by growth-factor withdrawal (Pierce *et al.*, 2008). Studying migratory GnRH neurons during embryogenesis in mice deleted for *Axl* (*Axl*^{-/-}), *Tyro3* (*Tyro3*^{-/-}) or both *Axl* and *Tyro3* (*Axl*^{-/-}, *Tyro3*^{-/-}) corroborated their roles *in vivo*. *Axl* and *Tyro3* deficiencies manifested later in adulthood in delayed sexual maturation and irregular estrus cycles with a significantly prolonged proestrus phase - all known to be dependent on GnRH neuronal function (Pierce *et al.*, 2008). The simultaneous deletion of *Tyro3* and *Axl* resulted in increased apoptosis of E15 migratory GnRH neurons, which led to a diminution of GnRH neurons reaching the forebrain at E15 as well as reduced GnRH neuronal populations in adult females (Pierce *et al.*, 2008). These findings indicate a role for TAM receptors in embryonic GnRH neuronal migration and survival.

To understand whether the TAM cognate ligands GAS6 and/or PROS1 play a role in GnRH neuronal migration *in vivo*, a first inspection was attempted in *Gas6*^{-/-} mice. Salian-Mehta and colleagues report that *Gas6* null females show a delayed vaginal opening and delayed first estrus, although normal estrus cycles were finally obtained later in adulthood (Salian-Mehta *et al.*, 2014). *Gas6* KO females had reduced numbers of GnRH neurons and their accumulation in early migratory locations at E15 suggests failed continued migration in the absence of Gas6. It is worthy to note that *Gas6* KO mice showed an overall improved phenotype compared to *Tyro3*; *Axl* double knockout (DKO) mice. The normal estrus cyclicity and the modest, yet significant phenotypes described

in *Gas6^{-/-}* females may suggest an *in vivo* role for PROS1 in GnRH development, as both GAS6 and PROS1 are expressed during GnRH neuronal development. While GAS6 expression did not differ between migratory and post-migratory GnRH neurons, that of PROS1 was found to be dynamically upregulated in post-migratory GnRH neurons (Pierce *et al.*, 2008). Whether PROS1 plays a role in GnRH neuronal development *in vivo* remains to be investigated.

Less is known about the role of TAMs in development of the male reproductive system. The first indication of TAM function in male (and female) fertility was reported in 1999 by Lu and colleagues (Lu *et al.*, 1999), who revealed that both female ovaries and male testis were histologically abnormal. The triple TAM knockout males never sired offspring, indicating their sterility. This report also identified cell death in the granulosa cells associated with growing ovarian follicles.

The few reports on the role of TAM signaling in the male reproductive system are chiefly based on studies in TAM triple KO mice, and focused at the postnatal perturbed clearance of immature and defective spermatids by Sertoli cells, eventually leading to failed testicular homeostasis and male sterility (Lu *et al.*, 1999). The expression of all TAM receptors was found to be confined to Sertoli cells, while expression of both GAS6 and PROS1 ligands was first detected in Leydig cells, and later upregulated in Sertoli cells coinciding sexual maturation (Lu *et al.*, 1999). Postnatal spermatogenesis is deffective in TAM triple KO mice, with delayed production of mature sperm and increasingly elevated sperm deficiency as these mice mature. Sertoli cell death was observed in these mice, which led to germ cell death during their maturation phase, specifically in spermatids and spermatocytes (Lu *et al.*,

1999). Interestingly, these mice had normal blood testosterone levels and exhibited normal sexual behavior. Thus, it was hypothesized that TAMs are necessary for Sertoli cells to provide trophic support for the developing spermatogonia. Additionally, the life-long continuous generation of sperm also requires the nonstop phagocytic clearance of apoptotic germ cells and cytoplasm extruded during sperm maturation. This rapid clearance of cell debris is performed by Sertoli cells, (Lu *et al.*, 2010), and is mediated by TAM receptors (Chen *et al.*, 2009; Lemke, Burstyn-Cohen, 2010; Lu *et al.*, 2010; Xiong *et al.*, 2008). GAS6 was shown to mediate Sertoli cell phagocytosis of apoptotic cells *in vitro* (Xiong *et al.*, 2008), however the viability of Sertoli cells in *Gas6^{-/-}* mice and their fertility suggests that PROS1, also expressed by Leydig cells may function redundantly *in vivo*. Indeed, MERTK stimulation by PROS1 is sufficient for normal apoptotic cell clearance in the testis, as revealed by a systematic genetic analysis in various TAM single and double mutant mice (Lew *et al.*, 2014).

Sertoli cell progenitors are already present within the developing gonad at E11.5, and were shown to play key roles in further testicular development, including testis cord formation, testicular vasculogenesis, Leydig cell differentiation and function, and the maintenance of undifferentiated spermatogonia (reviewed in Cool *et al.*, 2012). Sertoli cells also play a central role in the continuing prepubertal postnatal development of the testis, including in the postnatal development of Leydig cells, as was recently shown by the postnatal ablation of Sertoli cells (Rebourcet *et al.*, 2014). Whether TAM receptors are expressed by early Sertoli cells or whether they play a role in these embryonic or postnatal stages of testis development has not yet been studied.
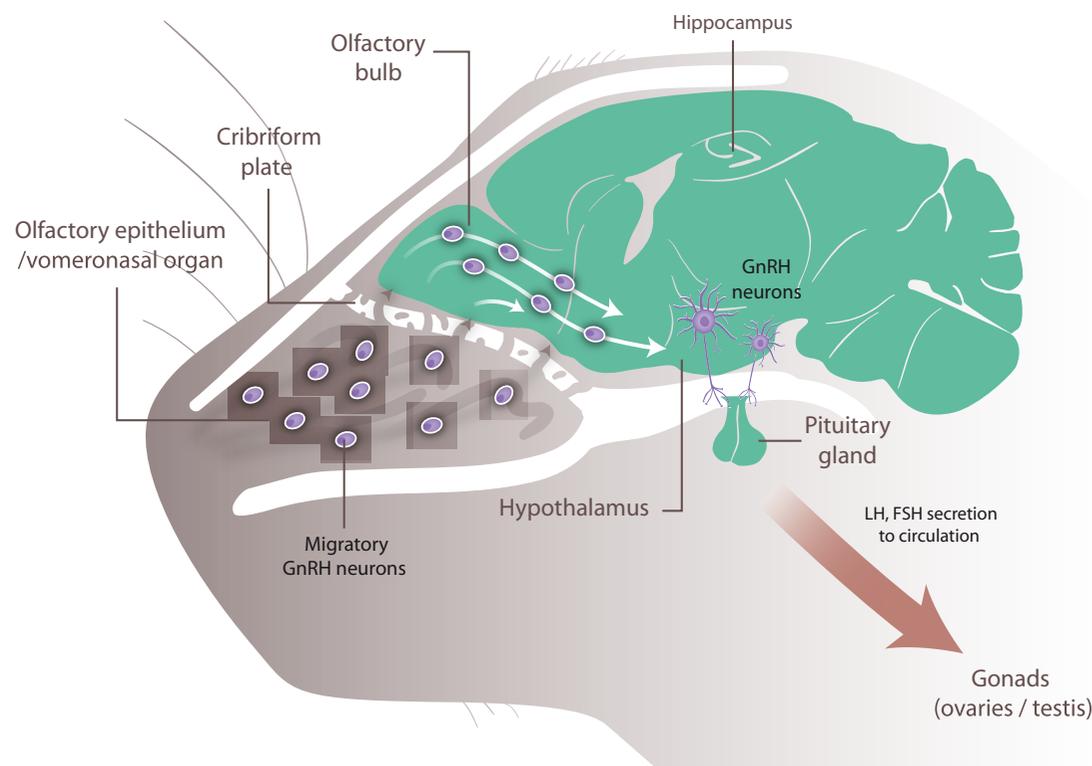

Fig. 2. TAM signaling and GnRH neuronal migration. *Gonadotropin-releasing hormone (GnRH) neurons are born in the olfactory placode and epithelium / vomeronasal organ. Nascent GnRH neurons migrate along the vomeronasal/olfactory nerve into the olfactory bulb through perforations at the cribiform plate. After entering the forebrain, GnRH neurons change their migratory trajectory and turn caudally, continue migration until they reach their final destination at the hypothalamic region. GnRH neurons release GnRH through their extended projections to the portal system of the pituitary gland, where they regulate secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into the circulation. GAS6 supports GnRH neuronal survival through AXL and TYRO3, which are expressed by migratory GnRH neurons. Mertk expression is upregulated in post-migratory GnRH neurons. Increased apoptosis and delayed migration with a reduction in GnRH neurons reaching their final destination is seen in Tyro3^{-/-} Axl^{-/-} double knockout mice (Pierce et. al., 2008).*

TAM receptor function in the developing nervous system

Among TAM receptors, TYRO3 is most prominently expressed in the nervous system, both during development and throughout adulthood. An extensive analysis of TAM receptor expression was performed in the rat developing brain, where TYRO3 expression was found to be relatively highly expressed in the periventricular neuroepithelial layers (subventricular zones) along the forebrain, midbrain and hindbrain regions, in the nasal olfactory epithelium and along the developing spinal cord (Prieto *et al.*, 2000). These brain regions are areas where active proliferation takes place during embryonic brain development and in a more restricted manner in neurogenic niches during adulthood (Zhao *et al.*, 2008). The complex regulation of neural stem cell (NSC) biology involves cellular and environmental factors, and is a multi-step process, where NSCs have several binary choice points. For example, stem cells are either quiescent or proliferating. Quiescent stem cells engage in proliferation upon internal or external signals. Once proliferating, stem cells divide to produce more stem cells, thus maintaining the stem cell pool through a process of self-renewal, while other daughter cells engage in differentiation. The differentiating cell again faces a binary fate choice, as it can adopt a neural or glial fate. Thus, the balance between quiescence and proliferation; self-renewal or differentiation and final fate acquisition play important roles in tissue homeostasis, and are regulated by complex molecular cues (Mu *et al.*, 2010).

In a transcriptomic screen Wang *et. al.* identified all three TAM receptors, and the ligand *Pros1* to be differentially enriched among neural stem/progenitor cells in the developing (E15.5) mouse cortex (Wang *et al.*, 2011). Indeed, AXL immune-expression was confined to the cortical ventricular zone at E15.5, where active proliferation takes place. Neutralization of *Axl* by *in-utero* electroporation of a dominant negative *Axl* construct caused cortical ventricular zone neural stem/progenitor cells to prematurely differentiate, and engage in migration towards the cortical plate. This premature differentiation was accompanied by an increase in the expression of the neuronal marker βIII-tubulin and a concomitant loss of the progenitor cell markers nestin, Pax6 and Tbr2 (Wang *et al.*, 2011). Analysis of BrdU labeling together with Ki67 immunoreactivity in cortices from Axl^{-/-};Mer^{-/-} DKOs also revealed the early exit of progenitor cells from the cell cycle and premature differentiation. Together, these results identified a role for TAM signaling in the maintenance of cortical embryonic neural progenitor cells.

Analysis of TAM triple knockout stem cells isolated from neonatal mouse forebrains supports a role for TAM receptors in maintaining neural progenitor identity, as well as providing trophic support for these cells (Ji *et al.*, 2014). When cultured, TAM triple knockout neural stem/progenitor cells expressed less nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) than control cells, suggesting trophic factor expression is regulated by TAM signaling. With respect to cell differentiation, while no effect was observed on glial daughter cell fate, a decrease in neuronal differentiation was observed in knockout cultured NSCs, suggesting TAM receptor signaling may be instructive for the acquisition of neuronal cell fates. However, deletion of all three TAM receptors was detrimental for *in vitro* culturing of neural stem/progenitor cells, which showed delayed growth profiles and elevated apoptotic rates compared to wild type cells (Ji *et al.*, 2014). Investigating the differential impact of each TAM receptor on NSC biology *in vivo* would be of great interest, but is currently hampered by the lack of genetic tools to selectively ablate TAM receptor expression within specific neurogenic cellular compartments. Thus, understanding the relative roles of *Tyro3*, *Axl* and *Mertk* specifically within NSCs versus other neurogenic niche brain cells such as microglia, endothelial cells and astrocytes is not yet possible *in vivo*. The recent generation of a mouse harboring a conditional *Mertk* allele (Fourgeaud *et al.*, 2016) would allow dissecting the relative role of *Mertk* in the developing nervous system.

In the adult, the postnatal ongoing generation of newborn neurons and glia from NSCs is critical for maintaining brain homeostasis and function. In rodents, NSCs residing in the subventricular zone (SVZ) provide newly generated neurons which migrate through the rostro-migratory stream and finally incorporate into the olfactory bulb. Neural stem cells residing in the subgranular hippocampal layer (SGZ) give rise to neurons which contribute to learning and memory (Deng *et al.*, 2010; Shors *et al.*, 2001). Adult murine SVZ neural stem/progenitor cells were shown to express all three TAM receptors and both ligands PROS1 and GAS6 (Gely-Pernot *et al.*, 2012). Gely-Pernot *et. al.* inspected the role of the TAM ligands GAS6 and PROS1 on adult SVZ neurogenesis. Cultured SVZ neural stem/progenitor cells isolated from GAS6^{-/-} mice showed reduced numbers of proliferating neural stem/progenitor cells compared to wild type control cells, and generated less self-renewing neurospheres, suggesting either impaired NSC proliferation, a reduction of the NSC pool, reduced self-renewal capacity, or a combination thereof. By contrast, antibody neutralization of PROS1 increased SVZ neural stem/progenitor cell proliferation *in vivo*, suggesting PROS1 is inhibitory for SVZ NSC proliferation *in vivo*, and counteracts GAS6 function (Gely-Pernot *et al.*, 2012).

The expression of TYRO3, MERTK, GAS6 and PROS1 in brain tissue is not confined to NSC/NPCs. Other cell types in the neurogenic niche which are known to influence neurogenesis, such as endothelial cells, microglia and astrocytes also express these TAM signaling components at steady state, and under inflammatory conditions. Thus, TAMs may influence NSC/NPC developmental processes in a NSC/NPC autonomous manner (which can be either autocrine or paracrine), or by an inter-cellular interaction, involving non- NSC/NPC cell types. For example, microglia regulate neurogenesis by secreting factors that affect different phases of the neurogenic process (Gemma *et al.*, 2013; Sato, 2015; Sierra *et al.*, 2014) and are also active in clearing of apoptotic newborn cells that are aborted during neurogenesis (Sierra *et al.*, 2010). Microglia also regulate neurogenesis under inflammatory conditions. Their polarized reaction to inflammation can lead either to a neuro-protective reaction positively supporting *de-novo* neurogenesis, or to the suppression of neurogenesis (Belarbi *et al.*, 2013).

Exclusive of *Tyro3*, all TAM signaling components are expressed by microglia (Butovsky *et al.*, 2014; Grommes *et al.*, 2008; Ji *et al.*, 2013), where they mediate anti-inflammatory immune signaling and phagocytosis (Grommes *et al.*, 2008). A recent work by Fourgeaud and colleagues showed that *Mertk* and *Axl* mediate the *in vivo* engulfment of apoptotic newborn neurons in the adult SVZ by microglia (Fourgeaud *et al.*, 2016), and that both ligands PROS1 and GAS6 function *in vitro* as MER agonists for this phagocytic event. Thus, TAM signaling contributes to the successful and productive process of adult neurogenesis by regulating the clearance of apoptotic newborn neurons. However, as mentioned above, the polarized response of microglia can also negatively

influence adult neurogenesis, through an elevated inflammatory response. Ji *et. al.* investigated microglia from TAM triple KO mice, and found they were characterized by a hyperactivated, pro-inflammatory cytokine profile in response to stimulation with LPS, CpG or poly(I:C) (Ji *et al.*, 2013). This hyper-inflammatory profile of microglia was neurotoxic and detrimental to adult neurogenesis both *in vitro* and *in vivo*. Elevated microglial IL-6 secretion was a key player in this effect, as knockout of the IL-6 gene in TAM triple KO mice restored hippocampal NSC/NPC proliferation and neurogenesis, as measured by newborn DCX$^+$ neurons (Ji *et al.*, 2013). Similar to microglia, TAM$^{-/-}$ astrocytes also secreted higher pro-inflammatory cytokines following stimulation by LPS, hampering NSC/NPC proliferation and the generation of newborn DCX+ neurons. Thus, microglia and astrocytes provide another facet by which TAM signaling modulates neurogenesis in a non NSC/NPC autonomous manner.

Taken together, TAM signaling plays a role in NSC/NPC proliferation and differentiation choice points during embryonic and postnatal neural development. While the role of TAM signaling in developmental and non-developmental processes builds upon investigating mice in which TAM receptors or GAS6 gene function was inactivated, very little is known about the role of PROS1 in this process. We recently conditionally ablated *Pros1* expression in neural stem/progenitor cells to investigate the relative contribution of PROS1 in stem/progenitor proliferation and differentiation. Active research is directed to better understand the role of PROS1 in neural stem/progenitor endogenous mechanisms and in niche cells. During the writing of this review, our group has published two studies identifying PROS1 as a multilevel regulator of NSC biology at the binary choice points of quiescence/ proliferation and self-renewal/differentiation, also impacting daughter cell fate. (Zelentsova *et al.,* 2017; Zelentsova-Levitskyi *et al .,* 2017). It is possible that activation of TAMs by the different ligands may elicit diverse downstream signaling pathways, differentially affecting different choice points along the neurogenic process.

TAM receptor function in the developing immune system

Most of the current knowledge on TAM signaling in the immune system is focused at the functional level. Adult mice lacking all three TAM receptors were first observed to develop chronic inflammation and autoimmunity, with constitutively activated macrophages (Camenisch *et al.*, 1999; Lemke *et al.*, 2003; Lu, Lemke, 2001). Further studies identified TAMs are pleiotropic negative regulators of innate immunity, attenuating the immune response in Dendritic cells (DCs) (Rothlin *et al.*, 2007). More recently, it was shown that T cell-derived PROS1 restrains DC activation via TAMs, revealing TAM function at the regulatory interface of the adoptive and innate immune arms (Carrera Silva *et al.*, 2013). These roles of TAMs were recently extensively reviewed by Rothlin and colleagues (Rothlin *et al.*, 2015). The involvement of TAMs in the development and maturation of immune cells is described below.

To date, TAMs were found to govern the development and maturation of natural killer (NK) cells, erythrocytes, and Langerhans cells (LC). The differentiation and maturation of committed NK progenitors involves the sequential and graduate acquisition and expression of functional surface markers and receptors, while the expression of other markers are downregulated. These processes are initiated in the bone marrow and are driven by signals provided by bone marrow stromal cells. Roth and colleagues have shown that all three TAM receptors contribute to the development and maturation of NK cells (Caraux *et al.*, 2006). They have shown that all three TAM receptors are expressed by NK cells, and that TAM expression on NKs is critical in the formation of the full NK cell repertoire, including their functional maturation. Additionally, recombinant GAS6 and PROS1 promoted the growth and differentiation of NK precursors *in vitro*, indicating functional ligand-receptor interaction drives this process (Caraux *et al.*, 2006). These results suggest that bone marrow stromal cells which express both GAS6 and PROS1 are likely to drive TAM-dependent NK differentiation in vivo. The analysis of NK cell repertoire and maturation in Gas6 null mice will likely contribute to understanding the role of GAS6 in NK cell ontogeny. Similarly, using a stromal-specific cre-mediated deletion of *Pros1* is necessary to verify and positively identify a role for PROS1 *in vivo*.

Erythropoiesis – the generation of oxygen carrying red blood cells - from hematopoietic stem cells was shown to be regulated by GAS6 and AXL (Angelillo-Scherrer *et al.*, 2008; Tang *et al.*, 2009). With an average life span of 40 days in mice, the bone marrow and spleen are the major sites of ongoing erythropoiesis from hematopoietic stem cells (HSCs) in adult mice (Green, 1966). Angelillo-Sherrer and colleagues studied erythropoiesis in *Gas6$^{-/-}$* mice, and found a significant reduction in numbers of erythroid progenitors (both burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) in fetal liver and adult bone marrow. GAS6 expression by erythroblasts is induced by Erythropoietin (Epo), and this effect is AXL-dependent. GAS6 enhanced erythroblast cell survival and Epo downstream signaling, and suppressed cytokine signaling known to inhibit erythropoiesis (Angelillo-Scherrer *et al.*, 2008). Another study identified the expression of the erythropoiesis-related genes GATA-1 and Epo-R to be regulated by AXL and MER (Tang *et al.*, 2009).

It is interesting to compare the different expression patterns of TAM receptors and ligands between differentiating NK cells and erythrocytes with those of differentiating NSCs. Both immune and neural precursor populations reside within their specific niche, where they intimately interact with non-precursor niche cells, also expressing TAM signaling components. However, while NSC/NPCs and erythroblasts express both TAM receptors and at least one ligand, NK precursor cells express TAM receptors, while ligands are predominantly expressed by the stromal cells of their niche. This may underlie fundamental differences in maturation, differentiation and specification between both developmental systems, reflecting altered autonomous and non-autonomous TAM mechanisms. More broadly within the hematopoietic stem cell niche, TAM signaling components are expressed by hematopoietic stem cells and bone marrow stromal cells (Avanzi *et al.*, 1997; Graham *et al.*, 1994; Satomura *et al.,* 1998) and GAS6 was shown to support hematopoiesis *in vitro* (Dormady *et al.*, 2000). Thus, various aspects of hematopoiesis and erythropoiesis are regulated by TAM signaling.

Another immune cell population whose development and differentiation was shown to be TAM-driven are Langerhans cells (LCs). LCs comprise a subset of myeloid-derived dendritic cells which reside within the skin epidermis. LCs represent the exclusive antigen-presenting cells in this peripheral tissue, and regulate skin immune responses against pathogens, environmental insult and stress (Ginhoux *et al.*, 2010). LCs are derived from embryonic hematopoietic precursors, which populate the skin at late embryo-

genesis. In the adult, under steady state conditions epidermal LCs leave the skin and migrate to draining lymph nodes, whereas new LCs repopulate the skin either from circulating monocytes, or by local proliferation. Under acute inflammatory conditions, LCs are repopulated by circulating precursors, mainly blood monocytes (Ginhoux, Merad, 2010).

TGF-β is a key regulator of LC development and maintenance, as TGF-β deficient mice lack DCs, (Borkowski *et al.*, 1996) and the conditional ablation of TGF-β receptor 1 in adult DCs results in their premature spontaneous maturation, indicating its importance in maintaining a pool of immature LCs in the epidermis (Kel *et al.*, 2010). In a recent report, Bauer and colleagues identified AXL to be a key effector of TGF-β1 during LC cell differentiation (Bauer *et al.*, 2012). Using a defined *in vitro* serum-free LC differentiation model, they have found that *AXL* transcript expression is induced during the early phases of human LC commitment, and that AXL expression was dependent on TGF-β1 induction. Similarly, TGF-β1 stimulated AXL expression in mouse bone marrow-derived DCs (BMDCs). Moreover, LC subsets in which AXL expression was upregulated showed an elevated differentiation potential compared to AXL-negative subsets. Of note, the diminution of 50% of LC cells in mice was observed only in TAM triple KO mice, as inactivation of *Axl* alone in mice did not result in diminution of LC numbers, which was only observed in human cells (Bauer *et al.*, 2012). This difference observed between human and murine LCs may be due to functional redundancy in mice. Alternatively, it may suggest that TAM signaling may be important for the maintenance of LCs in the epidermis. Understanding these differences requires further investigation.

Importantly, since AXL induction occurs in activated dendritic cells downstream of TLR signaling (Rothlin *et al.*, 2007), Bauer *et. al.* have also made the distinction between AXL activation during NK differentiation versus following TLR stimulation. Interestingly, TLR-mediated AXL induction occurs in the presence of TGF-β inhibitors, indicating context-specific and independent pathways for AXL activation in LCs. Finally, the ability of TGF-β to independently regulate all three TAM receptors in DCs and LCs (Bauer *et al.*, 2012; Rothlin *et al.*, 2007) is supported by immuno-histochemical staining which revealed the abundant expression of both GAS6 and PROS1 in human epidermis (Bauer *et al.*, 2012).

TAM receptor function in the developing vasculature

Expression of *Axl* and *Mertk*, as well as both ligands *Pros1* and *Gas6* by endothelial cells (ECs) and vascular smooth muscle cells (VSMCs) is well documented (Benzakour *et al.*, 1995; Benzakour *et al.*, 2000; Burstyn-Cohen *et al.*, 2009; Fair *et al.*, 1986; Stitt *et al.*, 1995). In the adult, TAMs regulate various aspects of vascular biology, including vessel integrity (Burstyn-Cohen *et al.*, 2009; Miner *et al.*, 2015; Zhu *et al.*, 2010), and vascular remodeling in response to injury (Korshunov *et al.*, 2006).

The expression of TAMs by ECs and VSMCs suggests they may also be instructive in formation of new blood vessels, both in health and in disease. Inhibition of AXL in primary human endothelial cells impaired blood vessel formation both *in vitro* and *in vivo*, suggesting possible clinical implications not only during developmental processes, but also in cancer settings (Holland *et al.*, 2010; Holland *et al.*, 2005). Endothelial cell motility is a necessary step in blood vessel formation, another aspect of angiogenesis

where AXL was shown to mediate VEGF-A-induced EC migration, through Akt activation (Ruan *et al.*, 2012). Thus, AXL seems to mediate both migration and tube formation of endothelial cells. The regulation of angiogenesis is a highly complex and coordinated process, involving molecular and cellular mechanisms, to ensure an overall balanced process, and importantly to avoid the aberrant or over-production of blood vessels. In this respect GAS6/AXL and PROS1/MERTK signaling were reported to inhibit VEGF-A-induced chemotaxis and tube formation of ECs (Fraineau *et al.*, 2012; Gallicchio *et al.*, 2005), suggesting TAM signaling may elicit both pro-and anti-angiogenic effects, contributing to an overall tightly regulated process. Interestingly, the inhibitory effect of both pathways was dependent on the tyrosine phosphatase SHP2.

With respect to TAM ligands - both ligands PROS1 and GAS6 promote survival of ECs and VSMCs (Burstyn-Cohen *et al.*, 2009; Gasic *et al.*, 1992; Healy *et al.*, 2001; Melaragno *et al.*, 2004; O'Donnell *et al.*, 1999). *Gas6-/-* mice are not reported to have abnormal angiogenesis (Angelillo-Scherrer *et al.*, 2001), albeit we have previously shown that *Gas6* null mice have compromised blood vessel integrity, as do *Axl-/-* mice, but not *Tyro3-/-* or *Mertk-/-* mice (Burstyn-Cohen *et al.*, 2009).

We previously reported a role for PROS1 in angiogenesis. *Pros1-/-* mice die embryonically between days E15.5-E17.5 from massive coagulopathy with associated hemorrhages, blood clots and defective blood vessel formation (Burstyn-Cohen *et al.*, 2009). At E13.5, prior to coagulation defects, *Pros1-/-* mice showed defects in blood vessel development and integrity not only in embryonic tissue *per-se*, but also in the extra-embryonic vasculature of the yolk sac. Endothelial cells in *Pros1-/-* mice presented with an activated morphology and aggregated in clusters but did not form tubular vessel structures, indicating an *in vivo* role for *Pros1* in angiogenesis. Consistent with *Pros1* being a mitogen for VSMCs (Gasic *et al.*, 1992), we found fewer VSMCs supporting blood vessels in *Pros1-/-* embryos. The few VSMCs that were found associated to nascent blood vessels did not form a supportive ring around the developing vasculature, leading to collapse of blood vessels. Interestingly, vascular development following the conditional knockout of *Pros1* from ECs (using the Tie2-cre) was not grossly affected, whereas deletion of *Pros1* from the VSMC cellular compartment (with SM22-Cre) led to leaky vasculature (Burstyn-Cohen *et al.*, 2009).

Finally, the bioactivity of PROS1 and GAS6 requires the vitamin K-dependent gamma-carboxylation of glutamate residues residing in the amino-terminal Gla domain (Fig. 1) (Lew *et al.*, 2014; Stitt *et al.*, 1995). Vitamin K epoxide reductase (VKOR) catalyzes the epoxide of vitamin K back to vitamin K, and is predominantly expressed in the vascular system. Studying the vitamin K pathway and its relation to angiogenesis can therefore highlight the importance of vitamin K-dependent proteins including PROS1 and GAS6 in angiogenesis, especially in light of the fact that warfarin which inhibits VKOR enzymatic activity by direct interaction (Tie *et al.*, 2008) is still the most commonly prescribed oral anticoagulant. The relevance of VKOR to the angiogenic process was demonstrated following stimulation and inhibition experiments. Overexpression of VKOR in human primary endothelial cells stimulated their proliferation, migration and tubular network formation, while VKOR knockdown inhibited EC tube formation (Wang *et al.*, 2005), further supporting the importance of this biochemical pathway. Moreover, a recent study identified the UBIAD-vitamin K2 gene to underlie

the cranial hemorrhages and defective EC survival during early vascular development, leading to vascular degeneration in the zebrafish mutant *reddish* (Hegarty *et al.,* 2013). Taken together, TAMs clearly play a role in vascular development and in angiogenesis, both in health and in disease. The relative roles of the different TAM signaling components remain to be revealed. Similarly, what are the relative contributions of PROS1 and GAS6 in ECs versus VSMCs? What downstream signaling and cellular events are induced by each possible receptor-ligand interaction, and how are these regulated? Answering these questions may provide a platform for the development of therapeutic manipulation for treatment of vascular diseases, or for controlling unrestrained angiogenesis as seen in cancer and wet age-related macular degeneration (AMD).

Concluding remarks

As mentioned above, inactivation of TAM receptors and/or ligands leads to developmental deficits in various tissues. The regulation of cell survival, proliferation, and migration by TAMs in tumor cells is mirrored in development, as seen for GnRH neurons, NSC/NPC populations and endothelial cells. TAM signaling also seems to play a role in cell differentiation processes, as seen for erythrocytes and natural killer cells, neural stem/precursor cells and in maturation of germ cells. These processes most likely involve interactions between precursor cell populations and their niche cells. Similar interactions are thought to occur between cancer stem cells and their environment, which may induce their exit from dormancy. In this respect, GAS6 and MERTK were shown to regulate prostate cancer stem cell dormancy and survival in the bone marrow (Jung *et al.*, 2016; Shiozawa *et al.*, 2010).

The abundant expression of TAM signaling components not only by the cells they affect but also within neighboring cells, together with the fact that PROS1 and GAS6 are secreted molecules, encumbers analysis of receptor-ligand inter-cellular cross-talk, and urges the generation of experimental tools (such as conditional knockout mice) for *Tyro3*, *Axl* and *Gas6* to precisely target TAM molecules in specific cell populations. The generation of such tools is expected to better elucidate the specific roles and mechanisms by which TAM signaling components impact numerous systems and processes during development and throughout adulthood.

References

ALLEN, M.P., ZENG, C., SCHNEIDER, K., XIONG, X., MEINTZER, M.K., BELLOSTA, P., BASILICO, C., VARNUM, B., HEIDENREICH, K.A. and WIERMAN, M.E. (1999). Growth Arrest-Specific Gene 6 (Gas6)/Adhesion Related Kinase (Ark) Signaling Promotes Gonadotropin-Releasing Hormone Neuronal Survival via Extracellular Signal-Regulated Kinase (ERK) and Akt. *Molec. Endocrinol.* 13: 191-201.

ANDERSON, H.A., MAYLOCK, C.A., WILLIAMS, J.A., PAWELETZ, C.P., SHU, H. and SHACTER, E. (2003). Serum-derived protein S binds to phosphatidylserine and stimulates the phagocytosis of apoptotic cells. *Nat Immunol* 4: 87-91.

ANGELILLO-SCHERRER, A., BURNIER, L., FLORES, N., SAVI, P., DEMOL, M., SCHAEFFER, P., HERBERT, J.M., LEMKE, G., GOFF, S.P., MATSUSHIMA, G.K. *et al.,* (2005). Role of Gas6 receptors in platelet signaling during thrombus stabilization and implications for antithrombotic therapy. *J Clin Invest* 115: 237-246.

ANGELILLO-SCHERRER, A., BURNIER, L., LAMBRECHTS, D., FISH, R.J., TJWA, M., PLAISANCE, S., SUGAMELE, R., DEMOL, M., MARTINEZ-SORIA, E., MAXWELL, P.H. *et al.,* (2008). Role of Gas6 in erythropoiesis and anemia in mice. *J. Clin. Invest.* 118: 583-596.

ANGELILLO-SCHERRER, A., DE FRUTOS, P., APARICIO, C., MELIS, E., SAVI, P., LUPU, F., ARNOUT, J., DEWERCHIN, M., HOYLAERTS, M., HERBERT, J. *et al.,*

(2001). Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. *Nat Med* 7: 215-221.

AVANZI, G.C., GALLICCHIO, M., CAVALLONI, G., GAMMAITONI, L., LEONE, F., ROSINA, A., BOLDORINI, R., MONGA, G., PEGORARO, L., VARNUM, B. *et al.,* (1997). GAS6, the ligand of Axl and Rse receptors, is expressed in hematopoietic tissue but lacks mitogenic activity. *Exp. Hematol* 25: 1219-1226.

AXELROD, H. and PIENTA, K.J. (2014). Axl as a mediator of cellular growth and survival. *Oncotarget.* 5: 8818-8852.

BAUER, T., ZAGÓRSKA, A., JURKIN, J., YASMIN, N., KÖFFEL, R., RICHTER, S., GESSLBAUER, B., LEMKE, G. and STROBL, H. (2012). Identification of Axl as a downstream effector of TGF-β1 during Langerhans cell differentiation and epidermal homeostasis. *J. Exp. Med.* 209: 2033-2047.

BELARBI, K. and ROSI, S. (2013). Modulation of adult-born neurons in the inflamed hippocampus. *Front. Cell. Neurosci.* 7:145; doi: 10.3389/fncel.2013.00145

BENZAKOUR, O., FORMSTONE, C., RAHMAN, S., KANTHOU, C., DENNEHY, U., SCULLY, M.F., KAKKAR, V.V. and D N COOPER, D.N. (1995). Evidence for a protein S receptor(s) on human vascular smooth muscle cells. Analysis of the binding characteristics and mitogenic properties of protein S on human vascular smooth muscle cells. *Biochem J* 308: 481-485.

BENZAKOUR, O. and KANTHOU, C. (2000). The anticoagulant factor, protein S, is produced by cultured human vascular smooth muscle cells and its expression is up-regulated by thrombin. *Blood* 95: 2008-2014.

BORKOWSKI, T.A., LETTERIO, J.J., FARR, A.G. and UDEY, M.C. (1996). A Role for Endogenous Transforming Growth Factor β1 in Langerhans Cell Biology: The Skin of Transforming Growth Factor β1 Null Mice Is Devoid of Epidermal Langerhans Cells. *J. Exp. Med.* 184: 2417-2422.

BRAND, T.M., IIDA, M., STEIN, A.P., CORRIGAN, K.L., BRAVERMAN, C.M., LUTHAR, N., TOULANY, M., GILL, P.S., SALGIA, R., KIMPLE, R.J. *et al.,* (2014). AXL Mediates Resistance to Cetuximab Therapy. *Cancer Res.* 74: 5152-5164.

BURSTYN-COHEN, T., HEEB, M.J. and LEMKE, G. (2009). Lack of Protein S in mice causes embryonic lethal coagulopathy and vascular dysgenesis. *J Clin Invest* 119: 2942-2953.

BURSTYN-COHEN, T., LEW, E.D., TRAVES, P.G., BURROLA, P.G., HASH, J.C. and LEMKE, G. (2012). Genetic dissection of TAM receptor-ligand interaction in retinal pigment epithelial cell phagocytosis. *Neuron* 76: 1123-1132.

BUTOVSKY, O., JEDRYCHOWSKI, M.P., MOORE, C.S., CIALIC, R., LANSER, A.J., GABRIELY, G., KOEGLSPERGER, T., DAKE, B., WU, P.M., DOYKAN, C.E. *et al.,* (2014). Identification of a unique TGF-[beta]-dependent molecular and functional signature in microglia. *Nature Neurosci.* 17: 131-143.

CAMENISCH, T.D., KOLLER, B.H., EARP, H.S. and MATSUSHIMA, G.K. (1999). A novel receptor tyrosine kinase, Mer, inhibits TNF-alpha production and lipopolysaccharide-induced endotoxic shock. *J Immunol* 162: 3498-3503.

CARAUX, A., LU, Q., FERNANDEZ, N., RIOU, S., DI SANTO, J.P., RAULET, D.H., LEMKE, G. and ROTH, C. (2006). Natural killer cell differentiation driven by Tyro3 receptor tyrosine kinases. *Nat Immunol* 7: 747-754.

CARRERA SILVA, E.A., CHAN, P.Y., JOANNAS, L., ERRASTI, A.E., GAGLIANI, N., BOSURGI, L., JABBOUR, M., PERRY, A., SMITH-CHAKMAKOVA, F., MUCIDA, D. *et al.,* (2013). T cell-derived protein S engages TAM receptor signaling in dendritic cells to control the magnitude of the immune response. *Immunity* 39: 160-170.

CHEN, J., CAREY, K. and GODOWSKI, P.J. (1997). Identification of Gas6 as a ligand for Mer, a neural cell adhesion molecule related receptor tyrosine kinase implicated in cellular transformation. *Oncogene* 14: 2033-2039.

CHEN, Y., WANG, H., QI, N., WU, H., XIONG, W., MA, J., LU, Q. and HAN, D. (2009). Functions of TAM RTKs in regulating spermatogenesis and male fertility in mice. *Reproduction* 138: 655-666.

COOL, J., DEFALCO, T. and CAPEL, B. (2012). Testis formation in the fetal mouse: dynamic and complex de novo tubulogenesis. *Wiley Interdisciplinary Reviews: Dev. Biol.* 1: 847-859.

CROSIER, P.S., LEWIS, P.M., HALL, L.R., VITAS, M.R., MORRIS, C.M., BEIER, D.R., WOOD, C.R. and CROSIER, K.E. (1994). Isolation of a receptor tyrosine kinase (DTK) from embryonic stem cells: structure, genetic mapping and analysis of expression. *Growth Factors* 11: 125-136.

CUMMINGS, C.T., DERYCKERE, D., EARP, H.S. and GRAHAM, D.K. (2013). Molecular pathways: MERTK signaling in cancer. *Clin. Cancer Res.* 19: 5275-5280.

D'CRUZ, P.M., YASUMURA, D., WEIR, J., MATTHES, M.T., ABDERRAHIM, H., LAVAIL, M.M. and VOLLRATH, D. (2000). Mutation of the receptor tyrosine kinase gene

Mertk in the retinal dystrophic RCS rat. *Hum Mol Genet* 9: 645-651.

DENG, W., AIMONE, J.B. and GAGE, F.H. (2010). New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? *Nature Rev. Neurosci.* 11: 339-350.

DORMADY, S.P., ZHANG, X.-M. and BASCH, R.S. (2000). Hematopoietic progenitor cells grow on 3T3 fibroblast monolayers that overexpress growth arrest-specific gene-6 (GAS6). *Proc. Natl. Acad. Sci. USA* 97: 12260-12265.

DUNCAN, J.L., LAVAIL, M.M., YASUMURA, D., MATTHES, M.T., YANG, H., TRAUT-MANN, N., CHAPPELOW, A.V., FENG, W., EARP, H.S., MATSUSHIMA, G.K. *et al.,* (2003). An RCS-Like Retinal Dystrophy Phenotype in Mer Knockout Mice. *Invest Ophthalmol Vis Sci* 44: 826-838.

ELKABETS, M., PAZARENTZOS, E., JURIC, D., SHENG, Q., PELOSSOF, R.A., BROOK, S., BENZAKEN, A.O., RODON, J., MORSE, N., YAN, J.J. *et al.,* (2015). AXL mediates resistance to PI3Kalpha inhibition by activating the EGFR/PKC/mTOR axis in head and neck and esophageal squamous cell carcinomas. *Cancer Cell* 27: 533-546.

ELLIOTT, M.R. and RAVICHANDRAN, K.S. (2010). Clearance of apoptotic cells: implications in health and disease. *J Cell Biol* 189: 1059-1070.

FAIR, D.S., MARLAR, R.A. and LEVIN, E.G. (1986). Human endothelial cells synthesize protein S. *Blood* 67: 1168-1171.

FANG, Z., XIONG, X., JAMES, A., GORDON, D.F. and WIERMAN, M.E. (1998). Identification of Novel Factors That Regulate GnRH Gene Expression and Neuronal Migration. *Endocrinology* 139: 3654-3657.

FOURGEAUD, L., TRAVÉS, P.G., TUFAIL, Y., LEAL-BAILEY, H., LEW, E.D., BURROLA, P.G., CALLAWAY, P., ZAGÓRSKA, A., ROTHLIN, C.V., NIMMERJAHN, A. *et al.,* (2016). TAM receptors regulate multiple features of microglial physiology. *Nature* 532: 240-244.

FRAINEAU, S., MONVOISIN, A., CLARHAUT, J., TALBOT, J., SIMONNEAU, C., KANTHOU, C., KANSE, S.M., PHILIPPE, M. and BENZAKOUR, O. (2012). The vitamin K–dependent anticoagulant factor, protein S, inhibits multiple VEGF-A–induced angiogenesis events in a Mer- and SHP2-dependent manner. *Blood* 120: 5073-5083.

GALLICCHIO, M., MITOLA, S., VALDEMBRI, D., FANTOZZI, R., VARNUM, B., AVANZI, G.C. and BUSSOLINO, F. (2005). Inhibition of vascular endothelial growth factor receptor 2–mediated endothelial cell activation by Axl tyrosine kinase receptor. *Blood* 105: 1970-1976.

GASIC, G.P., ARENAS, C.P., GASIC, T.B. and GASIC, G.J. (1992). Coagulation factors X, Xa, and protein S as potent mitogens of cultured aortic smooth muscle cells. *Proc. Natl. Acad. Sci. USA* 89: 2317-2320.

GELY-PERNOT, A., CORONAS, V., HARNOIS, T., PRESTOZ, L., MANDAIRON, N., DIDIER, A., BERJEAUD, J.M., MONVOISIN, A., BOURMEYSTER, N., DE FRUTOS, P.G. *et al.,* (2012). An endogenous vitamin K-dependent mechanism regulates cell proliferation in the brain subventricular stem cell niche. *Stem Cells* 30: 719-731.

GEMMA, C. and BACHSTETTER, A.D. (2013). The role of microglia in adult hippocampus neurogenesis. *Front. Cell. Neurosci.* 7:229; doi:10.3389/fncel.2013.00229

GINHOUX, F. and MERAD, M. (2010). Ontogeny and homeostasis of Langerhans cells. *Immunol Cell Biol* 88: 387-392.

GODOWSKI, P.J., MARK, M.R., CHEN, J., SADICK, M.D., RAAB, H. and HAMMONDS, R.G. (1995). Reevaluation of the roles of protein S and Gas6 as ligands for the receptor tyrosine kinase Rse/Tyro 3. *Cell* 82: 355-358.

GRAHAM, D., DAWSON, T., MULLANEY, D., SNODGRASS, H. and EARP, H. (1994). Cloning and mRNA expression analysis of a novel human protooncogene, c-mer [published erratum appears in Cell Growth Differ 1994 Sep;5(9):1022]. *Cell Growth Differ* 5: 647-657.

GRAHAM, D.K., DERYCKERE, D., DAVIES, K.D. and EARP, H.S. (2014). The TAM family: phosphatidylserine-sensing receptor tyrosine kinases gone awry in cancer. *Nature Rev. Cancer* 14: 769-785.

GREEN E.W. (Ed.) (1966). *Biology of the laboratory mouse.* Dover publications inc., New York.

GROMMES, C., LEE, C.Y., WILKINSON, B.L., JIANG, Q., KOENIGSKNECHT-TAL-BOO, J.L., VARNUM, B. and LANDRETH, G.E. (2008). Regulation of microglial phagocytosis and inflammatory gene expression by Gas6 acting on the Axl/Mer family of tyrosine kinases. *J Neuroimmune Pharmacol* 3: 130-140.

HALL, M.O., OBIN, M.S., HEEB, M.J., BURGESS, B.L. and ABRAMS, T.A. (2005). Both protein S and Gas6 stimulate outer segment phagocytosis by cultured rat

retinal pigment epithelial cells. *Exp Eye Res* 81: 581-591.

HALL, M.O., PRIETO, A.L., OBIN, M.S., ABRAMS, T.A., BURGESS, B.L., HEEB, M.J. and AGNEW, B.J. (2001). Outer segment phagocytosis by cultured retinal pigment epithelial cells requires Gas6. *Exp Eye Res* 73: 509-520.

HEALY, A.M., SCHWARTZ, J.J., ZHU, X., HERRICK, B.E., VARNUM, B. and FARBER, H.W. (2001). Gas 6 promotes Axl-mediated survival in pulmonary endothelial cells. *Am. J. Physiol - Lung Cell. Molec. Physiol.* 280: L1273-L1281.

HEGARTY, J.M., YANG, H. and CHI, N.C. (2013). UBIAD1-mediated vitamin K2 synthesis is required for vascular endothelial cell survival and development. *Development* 140: 1713-1719.

HERBISON, A.E. (2016). Control of puberty onset and fertility by gonadotropin-releasing hormone neurons. *Nat Rev Endocrinol* 12: 452-466.

HOLLAND, S.J., PAN, A., FRANCI, C., HU, Y., CHANG, B., LI, W., DUAN, M., TORNEROS, A., YU, J., HECKRODT, T.J. *et al.,* (2010). R428, a selective small molecule inhibitor of Axl kinase, blocks tumor spread and prolongs survival in models of metastatic breast cancer. *Cancer Res* 70: 1544.

HOLLAND, S.J., POWELL, M.J., FRANCI, C., CHAN, E.W., FRIERA, A.M., ATCHISON, R.E., MCLAUGHLIN, J., SWIFT, S.E., PALI, E.S., YAM, G. *et al.,* (2005). Multiple Roles for the Receptor Tyrosine Kinase Axl in Tumor Formation. *Cancer Res.* 65: 9294-9303.

JI, R., MENG, L., JIANG, X., CVM, N.K., DING, J., LI, Q. and LU, Q. (2014). TAM Receptors Support Neural Stem Cell Survival, Proliferation and Neuronal Differentiation. *PloS one* 9: e115140.

JI, R., TIAN, S., LU, H.J., LU, Q., ZHENG, Y., WANG, X., DING, J. and LI, Q. (2013). TAM receptors affect adult brain neurogenesis by negative regulation of microglial cell activation. *J. Immunol.* 191: 6165-6177.

JUNG, Y., DECKER, A.M., WANG, J., LEE, E., KANA, L.A., YUMOTO, K., CACKOWSKI, F.C., RHEE, J., CARMELIET, P., BUTTITTA, L. *et al.,* (2016). Endogenous GAS6 and Mer receptor signaling regulate prostate cancer stem cells in bone marrow. *Oncotarget* 7: 25698 - 256711.

KEL, J.M., GIRARD-MADOUX, M.J.H., REIZIS, B. and CLAUSEN, B.E. (2010). TGF-β Is Required To Maintain the Pool of Immature Langerhans Cells in the Epidermis. *J. Immunol.* 185: 3248-3255.

KORSHUNOV, V.A., MOHAN, A.M., GEORGER, M.A. and BERK, B.C. (2006). Axl, A Receptor Tyrosine Kinase, Mediates Flow-Induced Vascular Remodeling. *Circ Res* 98: 1446-1452.

LAI, C., GORE, M. and LEMKE, G. (1994). Structure, expression, and activity of Tyro 3, a neural adhesion-related receptor tyrosine kinase. *Oncogene* 9: 2567-2578.

LAI, C. and LEMKE, G. (1991). An extended family of protein-tyrosine kinase genes differentially expressed in the vertebrate nervous system. *Neuron* 6: 691-704.

LEMKE, G. (2013). Biology of the TAM receptors. *CSH Perspec. Biol.* 5: a009076.

LEMKE, G. and BURSTYN-COHEN, T. (2010). TAM receptors and the clearance of apoptotic cells. *Ann. NY. Acad. Sci.* 1209: 23-29.

LEMKE, G. and LU, Q. (2003). Macrophage Regulation by Tyro3 family receptors. *Curr. Opin. Immunol.* 15: 31-36.

LEMKE, G. and ROTHLIN, C.V. (2008). Immunobiology of the TAM receptors. *Nat Rev Immunol* 8: 327-336.

LEW, E.D., OH, J., BURROLA, P.G., LAX, I., ZAGÓRSKA, A., TRAVÉS, P.G., SCHELSSINGER, J. and LEMKE, G. (2014). Differential TAM receptor-ligand-phospholipid interactions delimit differential TAM bioactivities. *eLife* 2014;3:e03385 - doi: 10.7554/eLife.03385

LU, Q., GORE, M., ZHANG, Q., CAMENISCH, T., BOAST, S., CASAGRANDA, F., LAI, C., SKINNER, M.K., KLEIN, R., MATSUSHIMA, G.K. *et al.,* (1999). Tyro-3 family receptors are essential regulators of mammalian spermatogenesis. *Nature* 398: 723-728.

LU, Q. and LEMKE, G. (2001). Homeostatic regulation of the immune system by receptor tyrosine kinases of the Tyro 3 family. *Science* 293: 306-311.

LU, Q., LI, Q. and LU, Q. (2010). Regulation of phagocytosis by TAM receptors and their ligands. *Front. Biol.* 5: 227-237.

MANFIOLETTI, G., BRANCOLINI, C., AVANZI, G. and SCHNEIDER, C. (1993). The protein encoded by a growth arrest-specific gene (gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Mol Cell Biol* 13: 4976-4985.

MARK, M.R., CHEN, J., HAMMONDS, R.G., SADICK, M. and GODOWSK, P.J. (1996). Characterization of Gas6, a member of the superfamily of G domain-containing

proteins, as a ligand for Rse and Axl. *J Biol Chem* 271: 9785-9789.

MELARAGNO, M.G., CAVET, M.E., YAN, C., TAI, L.K., JIN, Z.G., HAENDELER, J. and BERK, B.C. (2004). Gas6 inhibits apoptosis in vascular smooth muscle: role of Axl kinase and Akt. *J Mol Cell Cardiol* 37: 881-887.

MINER, J.J., DANIELS, B.P., SHRESTHA, B., PROENCA-MODENA, J.L., LEW, E.D., LAZEAR, H.M., GORMAN, M.J., LEMKE, G., KLEIN, R.S. and DIAMOND, M.S. (2015). The TAM receptor Mertk protects against neuroinvasive viral infection by maintaining blood-brain barrier integrity. *Nat Med* 21: 1464-1472.

MU, Y., LEE, S.W. and GAGE, F.H. (2010). Signaling in adult neurogenesis. *Curr Opin Neurobiol* 20: 416-423.

NAGATA, K., OHASHI, K., NAKANO, T., ARITA, H., ZONG, C., HANAFUSA, H. and MIZUNO, K. (1996). Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. *J Biol Chem* 271: 30022-30027.

NAKAMURA, Y.S., HAKEDA, Y., TAKAKURA, N., KAMEDA, T., HAMAGUCHI, I., MIYAMOTO, T., KAKUDO, S., NAKANO, T., KUMEGAWA, M. and SUDA, T. (1998). Tyro 3 receptor tyrosine kinase and its ligand, Gas6, stimulate the function of osteoclasts. *Stem Cells* 16: 229-238.

O'BRYAN, J.P., FRYE, R.A., COGSWELL, P.C., NEUBAUER, A., KITCH, B., PRO-KOP, C., ESPINOSA, R., LE BEAU, M.M., EARP, H.S. and LIU, E.T. (1991). axl, a transforming gene isolated from primary human myeloid leukemia cells, encodes a novel receptor tyrosine kinase. *Mol. Cell. Biol.* 11: 5016-5031.

O'DONNELL, K., HARKES, I.C., DOUGHERTY, L. and WICKS, I.P. (1999). Expression of receptor tyrosine kinase Axl and its ligand Gas6 in rheumatoid arthritis: evidence for a novel endothelial cell survival pathway. *Am J Pathol* 154: 1171-1180.

OHASHI, K., NAGATA, K., TOSHIMA, J., NAKANO, T., ARITA, H., TSUDA, H., SUZUKI, K. and MIZUNO, K. (1995). Stimulation of sky receptor tyrosine kinase by the product of growth arrest-specific gene 6. *J Biol Chem* 270: 22681-22684.

PIERCE, A., BLIESNER, B., XU, M., NIELSEN-PREISS, S., LEMKE, G., TOBET, S. and WIERMAN, M.E. (2008). Axl and Tyro3 Modulate Female Reproduction by Influencing Gonadotropin-Releasing Hormone Neuron Survival and Migration. *Molec. Endocrinol.* 22: 2481-2495.

POON, I.K.H., LUCAS, C.D., ROSSI, A.G. and RAVICHANDRAN, K.S. (2014). Apoptotic cell clearance: basic biology and therapeutic potential. *Nature Rev. Immunol.* 14: 166-180.

PRASAD, D., ROTHLIN, C.V., BURROLA, P., BURSTYN-COHEN, T., LU, Q., GARCIA DE FRUTOS, P. and LEMKE, G. (2006). TAM receptor function in the retinal pigment epithelium. *Mol Cell Neurosci* 33: 96-108.

PRIETO, A.L., WEBER, J.L. and LAI, C. (2000). Expression of the receptor protein-tyrosine kinases Tyro-3, Axl, and mer in the developing rat central nervous system. *J Comp Neurol* 425: 295-314.

REBOURCET, D., O'SHAUGHNESSY, P.J., PITETTI, J.-L., MONTEIRO, A., O'HARA, L., MILNE, L., TSAI, Y.T., CRUICKSHANKS, L., RIETHMACHER, D., GUILLOU, F. *et al.,* (2014). Sertoli cells control peritubular myoid cell fate and support adult Leydig cell development in the prepubertal testis. *Development* 141: 2139-2149.

ROTHLIN, C.V., CARRERA-SILVA, E.A., BOSURGI, L. and GHOSH, S. (2015). TAM Receptor Signaling in Immune Homeostasis. *Ann. Rev. Immunol.* 33: 355-391.

ROTHLIN, C.V., GHOSH, S., ZUNIGA, E.I., OLDSTONE, M.B. and LEMKE, G. (2007). TAM Receptors Are Pleiotropic Inhibitors of the Innate Immune Response. *Cell* 131: 1124-1136.

ROTHLIN, C.V. and LEMKE, G. (2010). TAM receptor signaling and autoimmune disease. *Curr Opin Immunol* 22: 740-746.

RUAN, G.X. and KAZLAUSKAS, A. (2012). Axl is essential for VEGF-A-dependent activation of PI3K/Akt. *EMBO J.* 31: 1692-1703.

SALIAN-MEHTA, S., XU, M., PIERCE, A., BLIESNER, B., TOBET, S. and WIERMAN, M.E. (2014). Loss of Growth arrest specific gene 6 (Gas6) results in altered GnRH neuron migration, delayed vaginal opening and sexual maturation in mice. *Molec. Cell. Endocrinol.* 393: 164-170.

SATO, K. (2015). Effects of Microglia on Neurogenesis. *Glia* 63: 1394-1405.

SATOMURA, K., DERUBEIS, A.R., FEDARKO, N.S., IBARAKI-O'CONNOR, K., KUZNETSOV, S.A., ROWE, D.W., YOUNG, M.F. and ROBEY, P.G. (1998). Receptor tyrosine kinase expression in human bone marrow stromal cells. *J. Cell. Physiol.* 177: 426-438.

SCOTT, R.S., MCMAHON, E.J., POP, S.M., REAP, E.A., CARICCHIO, R., COHEN, P.L., EARP, H.S. and MATSUSHIMA, G.K. (2001). Phagocytosis and clearance of apoptotic cells is mediated by MER. *Nature* 411: 207-211.

SHIOZAWA, Y., PEDERSEN, E.A., PATEL, L.R., ZIEGLER, A.M., HAVENS, A.M., JUNG, Y., WANG, J., ZALUCHA, S., LOBERG, R.D., PIENTA, K.J. *et al.,* (2010). GAS6/AXL Axis Regulates Prostate Cancer Invasion, Proliferation, and Survival in the Bone Marrow Niche. *Neoplasia (New York, NY)* 12: 116-127.

SHORS, T.J., MIESEGAES, G., BEYLIN, A., ZHAO, M., RYDEL, T. and GOULD, E. (2001). Neurogenesis in the adult is involved in the formation of trace memories. *Nature* 410: 372-376.

SIERRA, A., BECCARI, S., DIAZ-APARICIO, I., ENCINAS, J.M., COMEAU, S. and TREMBLAY, M.E. (2014). Surveillance, phagocytosis, and inflammation: how never-resting microglia influence adult hippocampal neurogenesis. *Neural plasticity* 2014: 610343.

SIERRA, A., ENCINAS, J.M., DEUDERO, J.J., CHANCEY, J.H., ENIKOLOPOV, G., OVERSTREET-WADICHE, L.S., TSIRKA, S.E. and MALETIC-SAVATIC, M. (2010). Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell Stem Cell* 7: 483-495.

STITT, T.N., CONN, G., GORE, M., LAI, C., BRUNO, J., RADZIEJEWSKI, C., MATTSSON, K., FISHER, J., GIES, D.R., JONES, P.F. *et al.,* (1995). The anticoagulation factor protein S and its relative, Gas6, are ligands for the Tyro 3/Axl family of receptor tyrosine kinases. *Cell* 80: 661-670.

TANG, H., CHEN, S., WANG, H., WU, H., LU, Q. and HAN, D. (2009). TAM receptors and the regulation of erythropoiesis in mice. *Haematologica* 94: 326-334.

TIE, J.K. and STAFFORD, D.W. (2008). Structure and Function of Vitamin K Epoxide Reductase. In *Vitamins & Hormones*, vol. Volume 78. Academic Press, pp.103-130.

WANG, J., ZHANG, H., YOUNG, A.G., QIU, R., ARGALIAN, S., LI, X., WU, X., LEMKE, G. and LU, Q. (2011). Transcriptome analysis of neural progenitor cells by a genetic dual reporter strategy. *Stem Cells* 29: 1589-1600.

WANG, Y., ZHEN, Y., SHI, Y., CHEN, J., ZHANG, C., WANG, X., YANG, X., ZHENG, Y., LIU, Y. and HUI, R. (2005). Vitamin K Epoxide Reductase: A Protein Involved in Angiogenesis. *Am. Assoc. Cancer Res.* 3: 317-323.

WIERMAN, M.E., KISELJAK-VASSILIADES, K. and TOBET, S. (2011). Gonadotropin-releasing hormone (GnRH) neuron migration: Initiation, maintenance and cessation as critical steps to ensure normal reproductive function. *Front. Neuroendocrinol.* 32: 43-52.

XIONG, W., CHEN, Y., WANG, H., WANG, H., WU, H., LU, Q. and HAN, D. (2008). Gas6 and the Tyro 3 receptor tyrosine kinase subfamily regulate the phagocytic function of Sertoli cells. *Reproduction* 135: 77-87.

YANAGITA, M., ISHIMOTO, Y., ARAI, H., NAGAI, K., ITO, T., NAKANO, T., SALANT, D.J., FUKATSU, A., DOI, T. and KITA, T. (2002). Essential role of Gas6 for glomerular injury in nephrotoxic nephritis. *J. Clin. Invest.* 110: 239-246.

ZAGORSKA, A., TRAVES, P.G., LEW, E.D., DRANSFIELD, I. and LEMKE, G. (2014). Diversification of TAM receptor tyrosine kinase function. *Nat Immunol* 15: 920-928.

ZELENTSOVA, K., TALMI, Z., ABBOUD-JARROUS, G., SAPIR, T., CAPUCHA, T., NASSAR, M., and BURSTYN-COHEN, T. (2016). Protein S regulates neural stem cell quiescence and neurogenesis. *Stem Cells* 35: 679-693.

ZELENSTOVA-LEVITSKYI, K., TALMI, Z., ABOUD-JARROUS, G., CAPUCHA, T., SAPIR, T., and BURSTYN-COHEN, T. (2017). Protein S negatively regulates neural stem cell self-renewal through Bmi-1 signaling. *Front. Mol NeuroSci.* 10.3389/fnmol.2017.00124.

ZHAO, C., DENG, W. and GAGE, F.H. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell* 132: 645-660.

ZHU, D., WANG, Y., SINGH, I., BELL, R.D., DEANE, R., ZHONG, Z., SAGARE, A., WINKLER, E.A. and ZLOKOVIC, B.V. (2010). Protein S controls hypoxic/ischemic blood-brain barrier disruption through the TAM receptor Tyro3 and sphingosine 1-phosphate receptor. *Blood* 115: 4963-4972.

Further Related Reading, published previously in the *Int. J. Dev. Biol.*

Expression of the DTK receptor tyrosine kinase during zebrafish development
M Jansa Perez, J A Walshe, K E Crosier and P S Crosier
Int. J. Dev. Biol. (1996) 40: S101-S102
http://www.intjdevbiol.com/web/paper/9087716

Sexual dimorphism in estrogen-induced synaptogenesis in the adult hippocampus
Nicola Brandt, Ricardo Vierk and Gabriele M. Rune
Int. J. Dev. Biol. (2013) 57: 351-356
http://dx.doi.org/10.1387/ijdb.120217gr

The spermatogonial stem cell niche in testicular germ cell tumors
Unai Silván, Alejandro Díez-Torre, Pablo Moreno, Jon Arluzea, Ricardo Andrade, Margarita
Silió and Juan Aréchaga
Int. J. Dev. Biol. (2013) 57: 185-195
http://dx.doi.org/10.1387/ijdb.130068ja

Identification of side population cells in mouse primordial germ cells and prenatal testis
Maria-Lucia Scaldaferri, Stefania Fera, Laura Grisanti, Massimo Sanchez, Mario Stefanini,
Massimo De Felici and Elena Vicini
Int. J. Dev. Biol. (2011) 55: 209-214
http://dx.doi.org/10.1387/ijdb.092977ms